FIG.1A

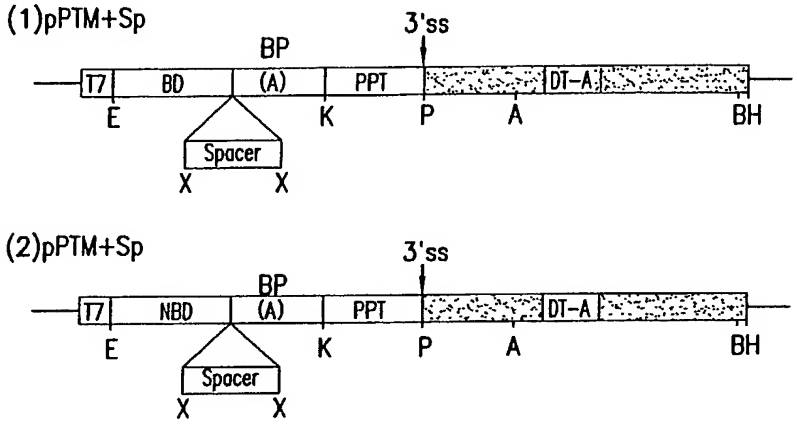

FIG.1B

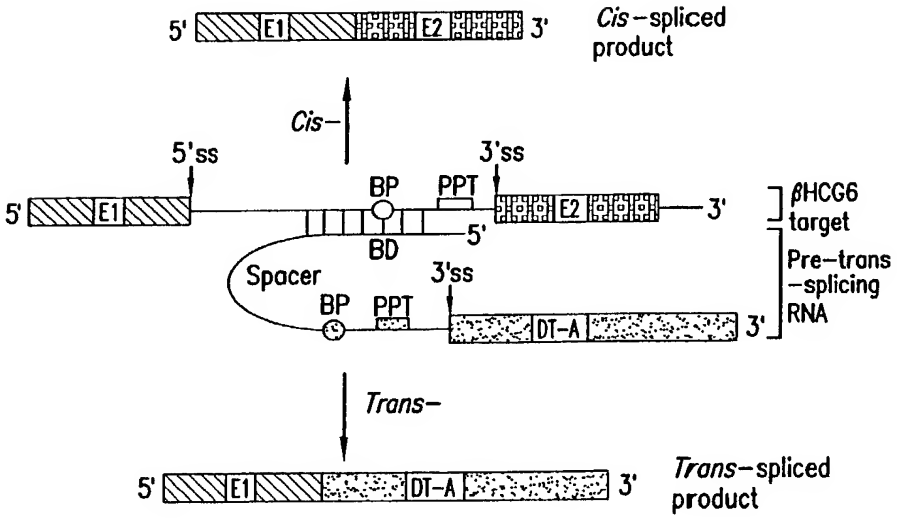

FIG.1C

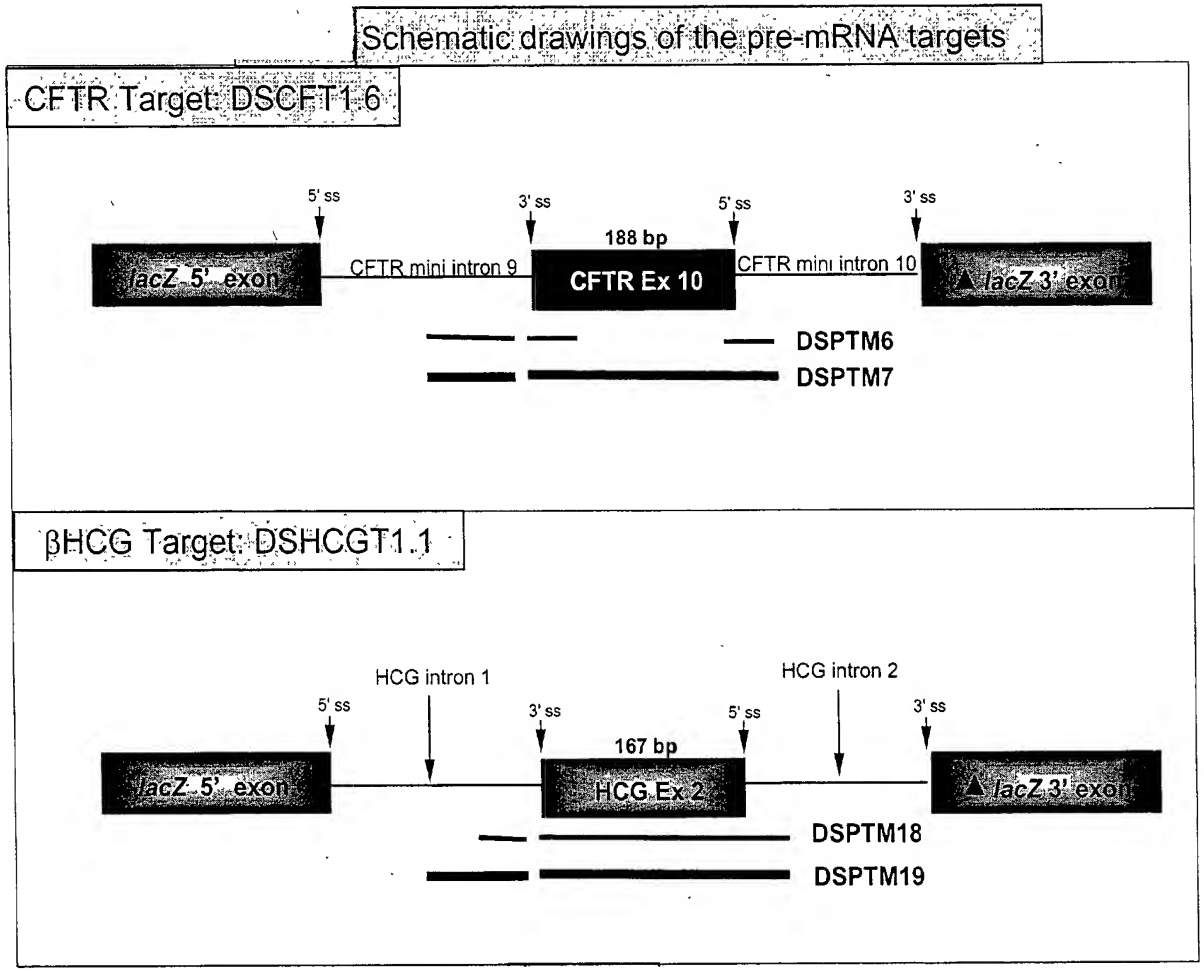

Figure 2

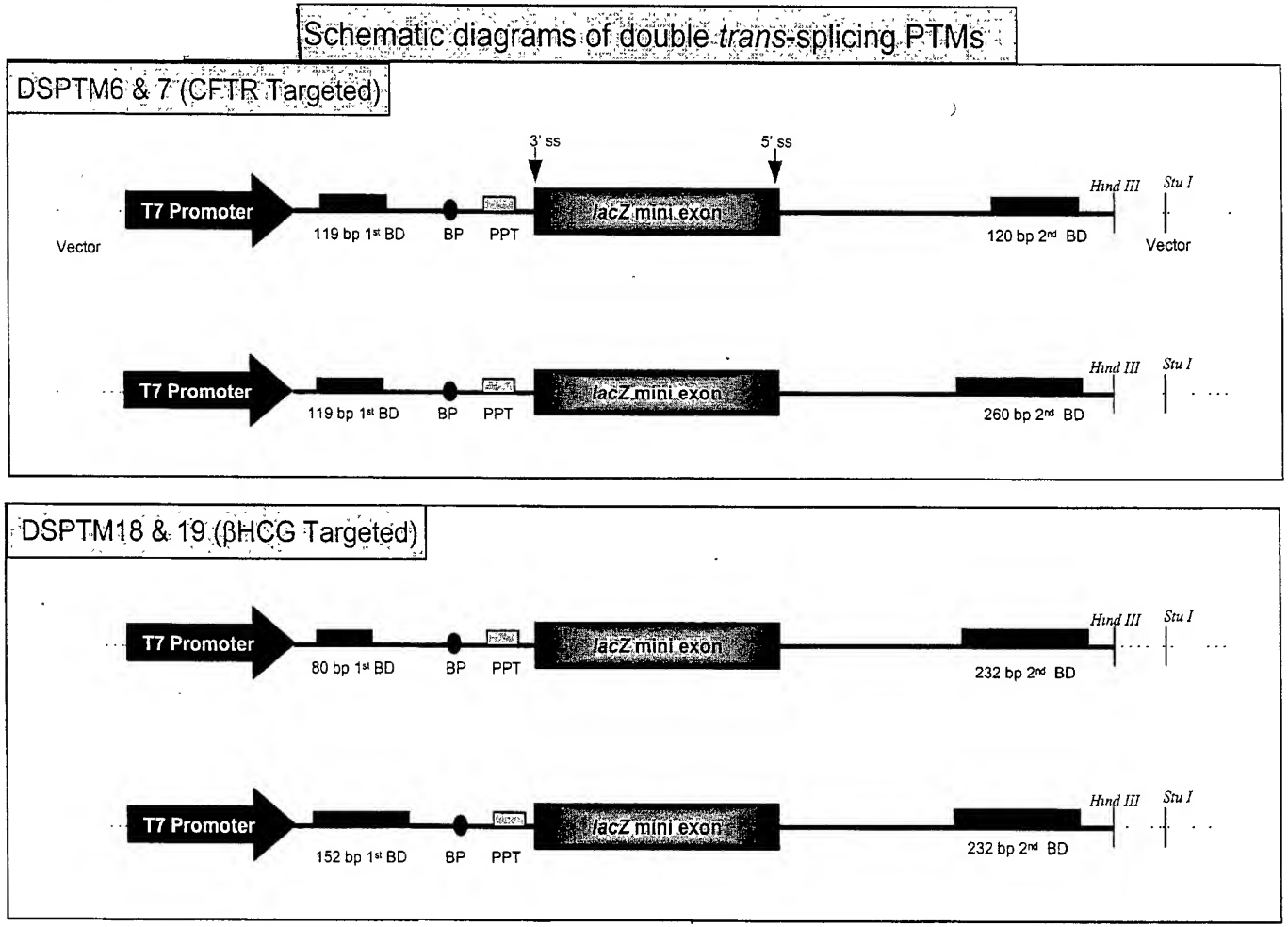

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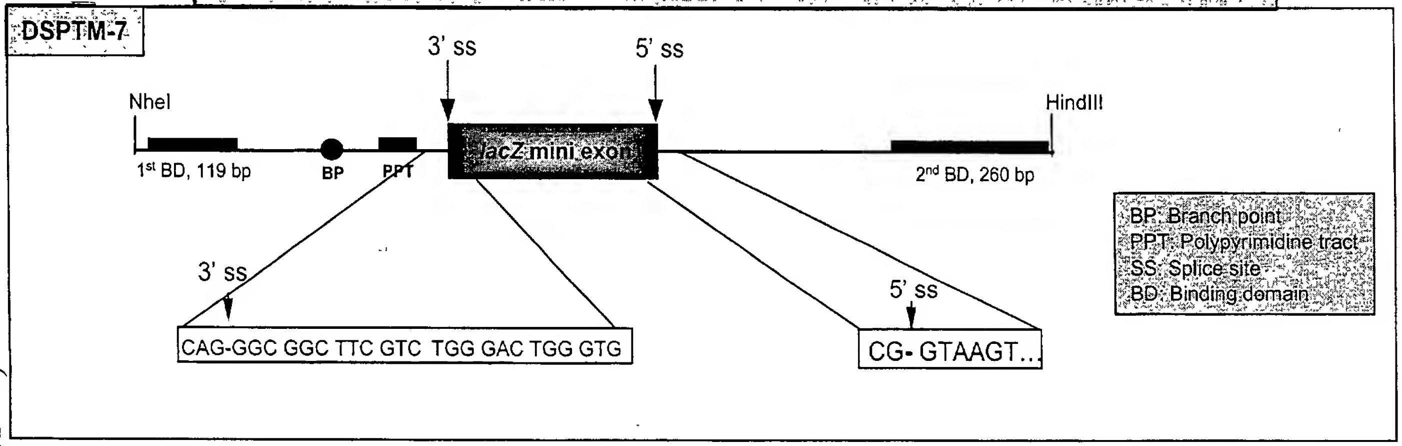
Figure 3

Diagram and important structural elements of double *trans*-splicing PTM7



1st BD (119 bp) : GATTCACTTGCTCCAATTATCATCCTAAGCAGAAGTGTATATTCTTATTTGTAAAGATTCTATTAACTCATTTGATTCAAAATA TTTAAAATACTTCCTGTTTCATACTCTGCTATGCAC

Spacer sequences: AACATTATTATAACGTTGCTCGAA

BP — PPT — 3' ss — *lacZ* mini exon

BP, PPT and acceptor splice site: TACTAAC T *GGTACC* TCTTCTTTTTTTTTTT *GATATC* CTGCAG <u>GGC GGC TTC GTC TGG GAC TGG</u>

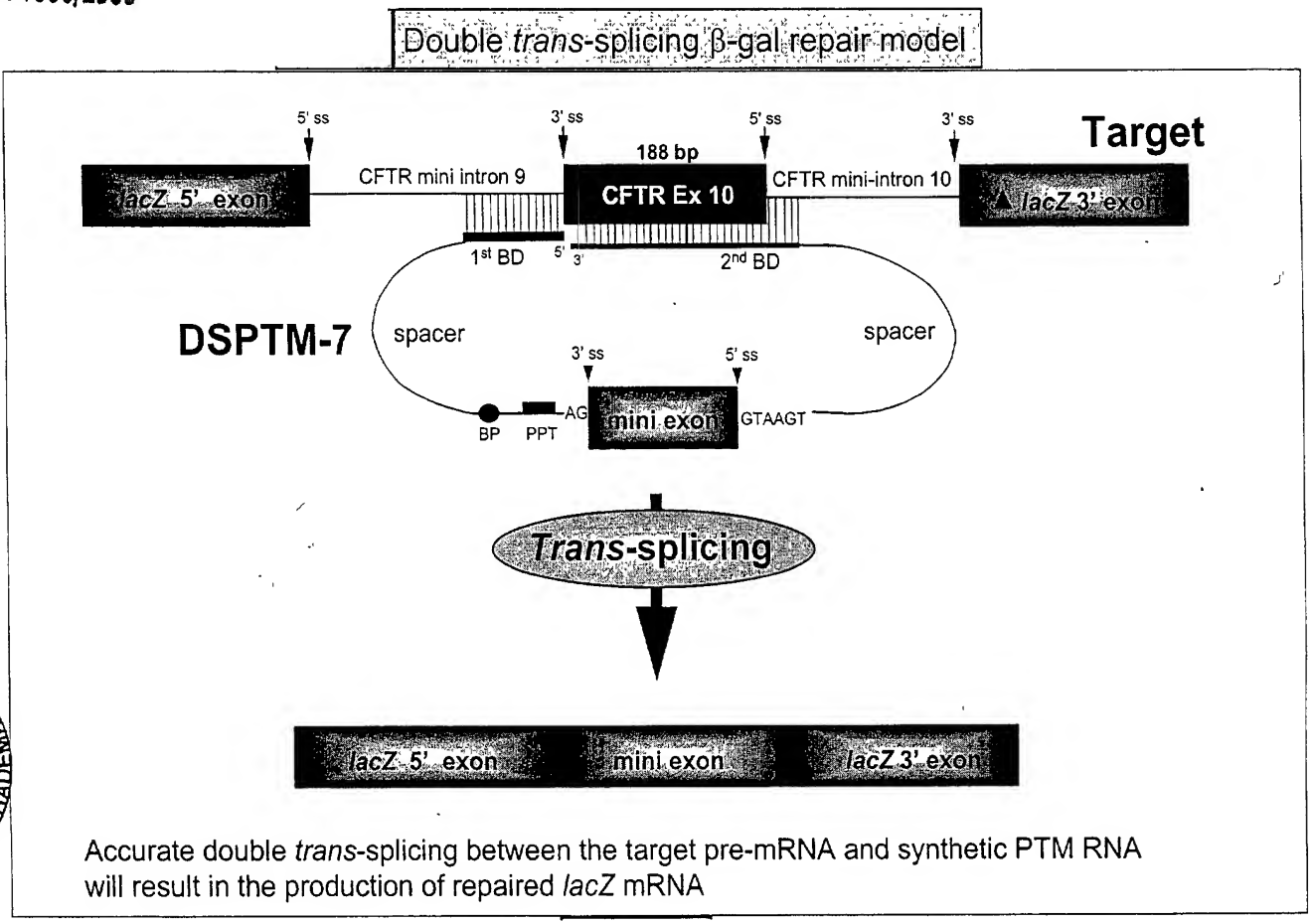
lacZ mini exon — 5' ss

5' donor site and 2nd spacer sequence: <u>TGA ACG</u> *GTAAGT* GTTATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACGCTAA GATCCACCGG

2nd BD (260 bp): TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTT*GAATTC*ATGCTTTGATGACGCTTCTGTATCTATATTCATCATTGGAA ACACCAATGATTTTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGTGCTTAA AAAAACCCTCT*GAATTC*TCCATTTCTCCCATAATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATTATTAACTCA TTATCAAATCACGC

Figure 4

Figure 5



Accurate double *trans*-splicing between the target pre-mRNA and synthetic PTM RNA will result in the production of repaired *lacZ* mRNA

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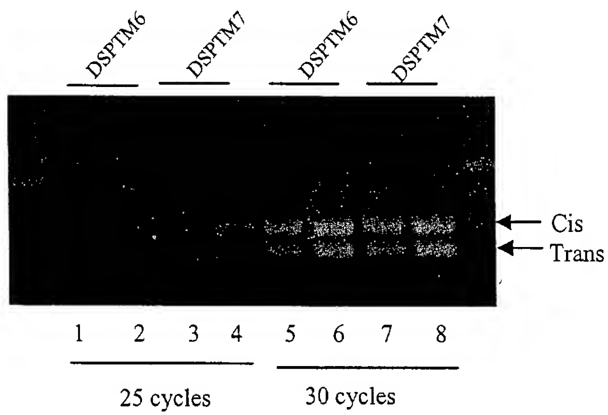
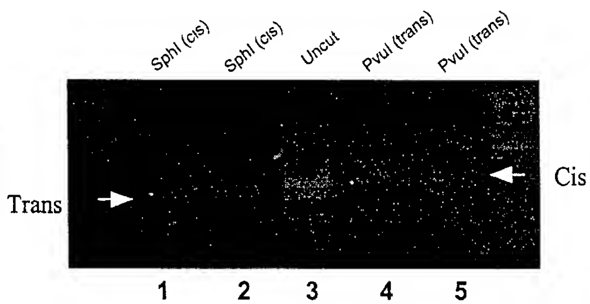
Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in 293T cells

2nd PCR Amplification



Diagnostic Test



DSPTM6 and 7 (CFTR targeted)

Methods

Transfect 293T cells with DSPTM6 and DSPTM7 *in vitro* transcribed, gel purified RNA (2.5-5.0 µg)

Isolate total RNA, cDNA synthesis (Lac6R), PCR amplification (20 cycls, KI-1F + Lac6R), digest with *Sph* I + *Dde* I (*cis*-specific) at 37°C/ON

Purify double *trans*-spliced product using Biotin-Lac21R probe

PCR amplify the captured *trans*-spliced product (KI-2F+Lac6R). Expected products: *cis*- 260bp; *trans*- 220 bp.

Diagnostic test: Digest PCR product with *Pvu* I (*trans*-specific) and with *Sph* I (*cis*-specific) at 37°C for 2-3 hr

Sequence to confirm the accuracy of double *trans*-splicing

Figure 6A

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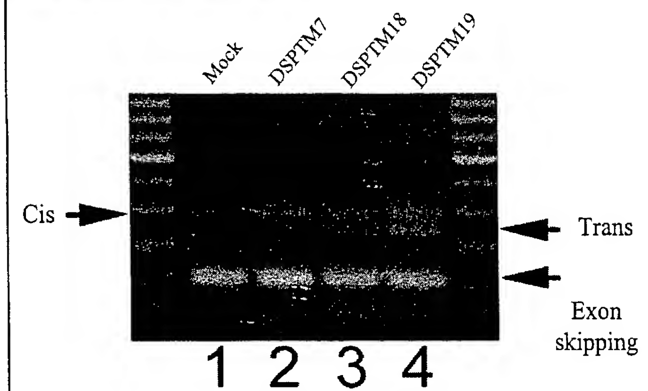
Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in stable cells

2nd PCR Amplification



DSPTM18 and 19 (HCG targeted)

Methods

Transfect DSHCGT1.1 stable cells with DSPTM7, DSPTM18 and DSPTM19 *in vitro* transcribed, gel purified RNA (2.5-5.0 µg)

Isolate total RNA, cDNA synthesis (Lac6R), PCR amplification (20 cycls, KI-1F + Lac6R), digest with *Sph* I + *Dde* I (*cis*-specific) at 37°C/ON

Purify double *trans*-spliced product using Biotin-Lac21R probe

PCR amplify the captured *trans*-spliced product (KI-2F + Lac6R). Expected products: *cis*- 260bp; *trans*- 220 bp

Sequence to confirm the accuracy of double *trans*-splicing

Figure 6B

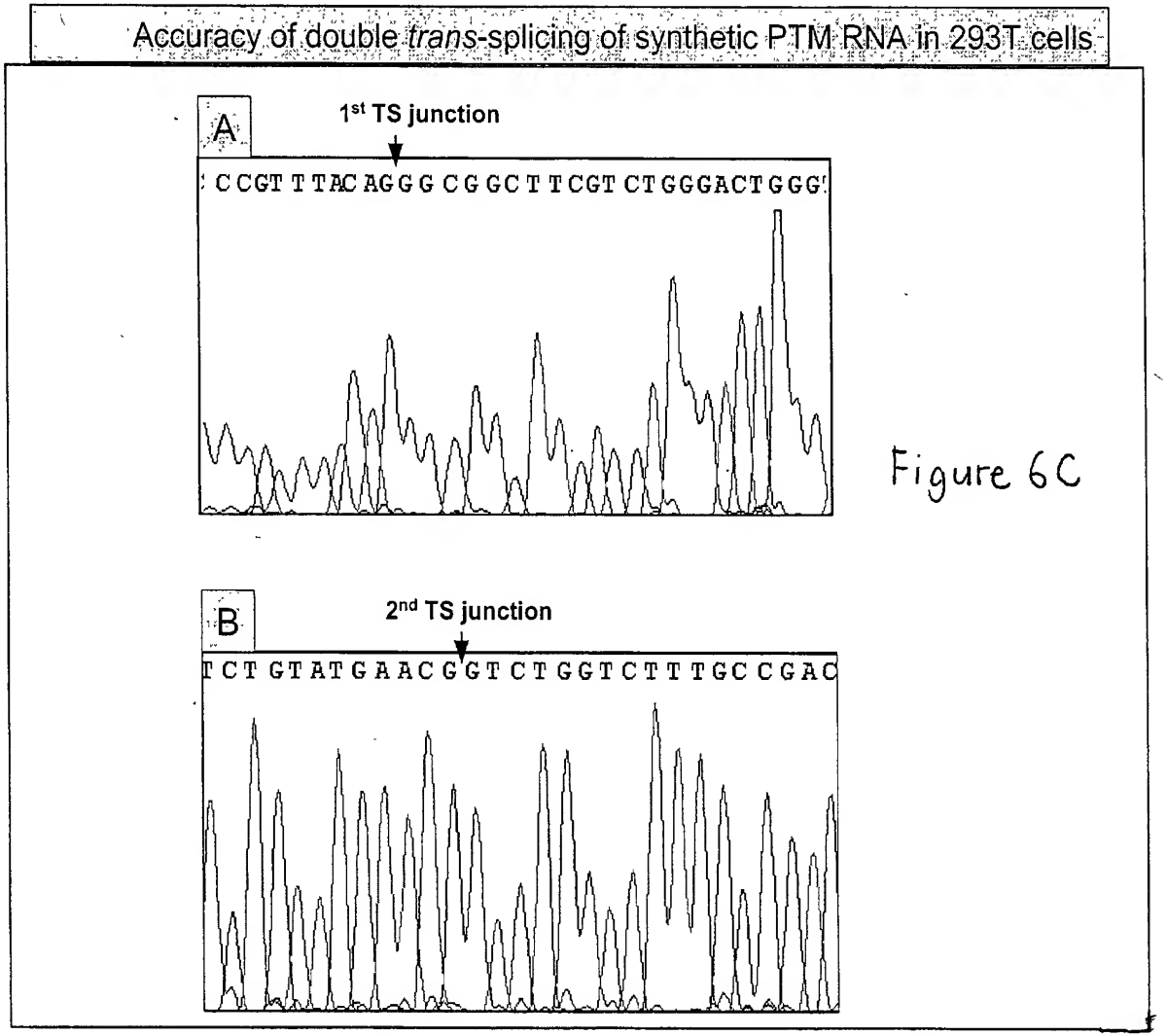


Figure 6C

Restoration of β-gal function through RNA transfection in 293T cells
(Proof-of-concept for SMaRT RNA Therapeutics!!)
Synthetic RNA, Double *trans*-splicing

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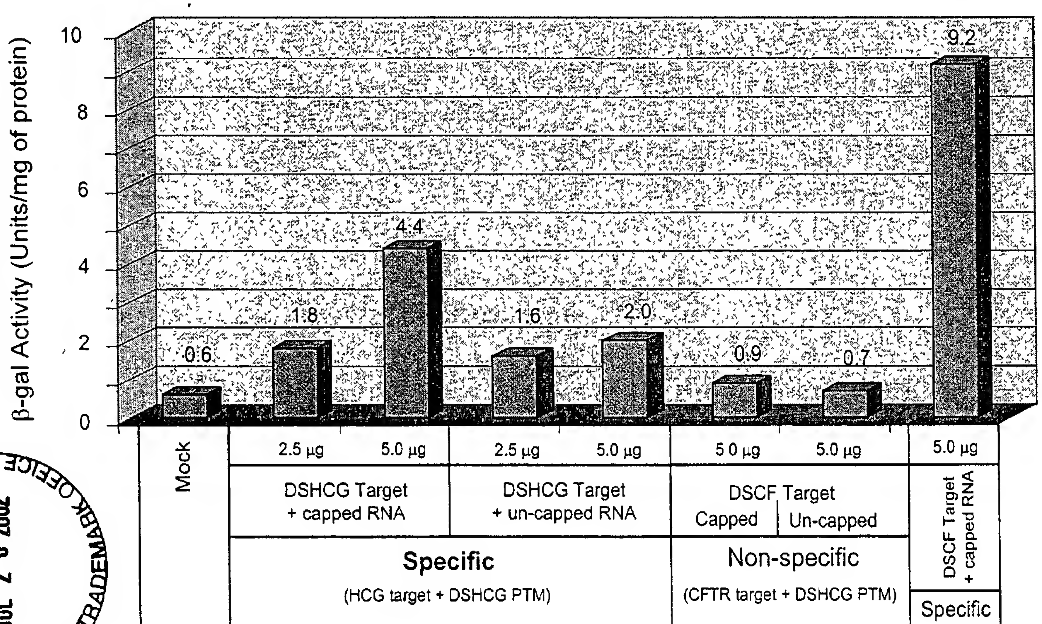

Methods

Transfect 293T cells
↓
Harvest cells after 48 hr
↓
Prepare cellular extract
↓
Assay for β-gal activity

Figure 7